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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/468,610	06/06/95	BURTON	S 010055-134

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EXAMINER	
WEBER, J	
ART UNIT	**PAPER NUMBER**
1651	20

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/468,610	Applicant(s) BURTON ET AL.	
	Examiner Jon P. Weber, Ph.D.	Art Unit 1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5 and 7-23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5 and 7-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Prosecution is hereby reopened in view of the new grounds of rejection set forth below. Claims 1-5 and 7-23 have been presented for examination.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 4-5, 10-16, 18, 20 and 22-23 are rejected under 35 U.S.C. 102(b) as being anticipated by Boardman et al. (1953).

A complex between an ion exchange resin and a target protein is claimed wherein the complex is formed at a pH value of between 5-9, where the resin is uncharged, and the target protein is bound to the resin by hydrophobic interactions. The ion exchange resin consists of a solid support matrix and a covalently attached ionizable ligand.

Boardman et al. (1953) disclose separation of proteins on ion exchange media with pH elution. At low pH the cation exchange media is uncharged and binds the proteins. As the pH is raised, the protein is eluted. Figure 1(a) illustrates the technique with cytochrome C on Amberlite IRC-50 [a cross-linked poly(methacrylic acid) with a capacity of 10 Meq/g]. At a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged. Between pH values of 6-7 the protein elutes from the column. At the same pH range, the affinity for sodium ions increases, corresponding to an ionization of the carboxylic groups on the resin.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-5 and 7-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957).

A complex between an ion exchange resin and a target protein is claimed wherein the complex is formed at a pH value of between 5-9, where the resin is uncharged, and the target protein is bound to the resin by hydrophobic interactions. The ion exchange resin consists of a solid support matrix and a covalently attached ionizable ligand. The resin may further comprise non-ionizable ligands.

The teachings of Boardman et al. (1953) have been discussed above. Boardman et al. (1953) lack the full range of resins that may be used.

Sasaki et al. (1979) disclose binding several enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. The resin can be eluted by increasing the pH so that the carboxyl groups dissociate with a concomitant loss of hydrophobicity and acquisition of a repulsive charge which in combination decreases the binding affinity of the bound enzymes. This process of using the Amberlite ion-exchange medium is termed hydrophobic-ionic chromatography. At page 1548,

the hydrophobic-ionic type of chromatography is defined as when the order of elution of proteins from the resin "is controlled by the remaining hydrophobic affinity plus the increased electrostatic affinity minus the increased electrostatic repulsion produced as the carboxyl groups are dissociated". Contrary to conventional ion exchange chromatography, enzymes bind to the uncharged functional groups and dissociate when the functional groups become charged. The acid base titration curve of Amberlite CG-50 shown in Fig. 1 demonstrates that below a pH value of about 4.5, the resin is fully protonated and therefore should exhibit no charge. Additionally, this figure demonstrates to a person of skill in the art how to determine the effective pH range of a given resin in the hydrophobic-ionic chromatography method. That is, the titration curve shows conditions when the protein will be bound by hydrophobic effects, and conditions when ionic effects will dissociate the protein.

The lack of ionic strength dependence on the binding of proteins at pH 4 to Amberlite CG-50 (page 1540, column 2) and elution of proteins with organic solvents (page 1546, column 2) is further evidence that the enzymes are bound to the ion exchange matrix by hydrophobic effects. Sasaki et al. (1979) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Sasaki et al. (1982) disclose binding several microbial enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. Subsequently, elution is effected by increasing pH to ionize the resin. This overall process is termed hydrophobic-ionic chromatography (abstract). In Figure 5 a cartoon is provided to explain the proposed mechanism of hydrophobic-ionic chromatography. The cartoon clearly indicates, in general terms, that with an acidic group, binding occurs below a certain pH

and desorption occurs above the critical pH. Different proteins having different interacting groups are released at different critical pH values (X or Y). This cartoon does not require any particular resin. It is a generalization of the concept of hydrophobic-ionic chromatography. The figure legend to Figure 5 clearly states, "in the case of Amberlite CG-50, X is 4.5". The figure legend continues to describe general mechanism, "with the use of appropriate adsorbent carrying alkaline groups, ... the relationship to pH would be opposite". While the cartoon illustrates the general principle with an ion-exchange resin that is acidic in nature, if the general mechanism is applied to an ion-exchange resin which is basic in nature, the effect of pH would be the opposite, i.e, lowering the pH would effect elution as the basic resin became charged. To understand this better, consider a resin with an amine functional group attached thereto. At sufficiently alkaline pH values, the resin is in the form of free amine and is uncharged. At lower pH values the basic amine becomes protonated to its conjugate acid and assumes a positive charge. Sasaki et al. (1982) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Kunin (1958) dislcoses titration curves of several ion exchange media and develops some of the mathematics of describing the dissociation. The well-known Henderson-Hasselbach equation is said to fit the titration data well. Accordingly, the pKa is the pH at which the ionizable group is half titrated. Figure 13 provides titration curves for Amberlite IRC-50 (used by Boardman et al., 1953) in water and in different concentrations of KCl. The pKa in water is about 8.5 and lower in the presence of KCl. At 2 pH units below the pKa, the ionized form of an acid comprises less than 0.1% of the total acid.

Topp et al. (1949) discloses the titration of several cases of ion exchange resins. Figure 2 shows the titration with poly(methacrylic acid). In the absence of added salt, the pKa is seen to

be about 8.5-9, while in the presence of 0.1 M NaCl, the pKa is lowered to about 7. In the absence of salt, exchange does not occur below pH value of 6.

Kitchener (1957) discloses that the carboxylic acid functionality normally titrates between 7 and 11 with a midpoint of about 9 which is lowered to about 7 in the presence of 0.1N KCl (based upon the data of Topp et al., 1949).

Guthrie (1957) lists the pH at half capacity (which corresponds to a phenomenological pKa) for a number of ion exchange cotton fabrics in Table I. Several of the modifying groups have pKa values in the range of 5-9.

A person of ordinary skill in the art at the time the invention was made would have been motivated to use ion exchange media to separate proteins where the proteins are bound at a pH value where the media is uncharged and then eluting by changing the pH to a value where the media is charged according to Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) because media which can be used in the claimed pH range of 5-9 are known in the art as demonstrated by Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957).

A wide range of ion-exchange media are known in the art and dozens of patents have been issued describing them. Media containing ionizable groups in concert with non-ionizable groups are well-known. Given the teachings of the art of record, it would constitute nothing more than routine optimization to select a suitable ion exchange media compatible with the target protein and having ionizable functional groups in the desired range of 5-9.

Hence, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to separate proteins with ion exchange media with a pKa value in the range of 5-9.

Other references cited by examiner but not relied upon are cited to establish the state of the art.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jon P. Weber, Ph.D. whose telephone number is 703-308-4015. The examiner can normally be reached on daily, off 1st Fri, 9/5/4.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 703-308-4743. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for regular communications and 703-308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Jon P. Weber, Ph.D.
Primary Examiner
Art Unit 1651

JPW
September 26, 2001

John J. Doll, Director
Technology Center 1600